Yield of canola (*Brassica napus* L.) benefits more from elevated CO_2 when access to deeper soil water is improved

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ABSTRACT

This study investigated the interactive effects of atmospheric CO_2 concentration ([CO_2]) and water availability on yield, root growth and water use of two canola cultivars with contrasting growth and vigour (vigorous hybrid cv. Hyola 50 and non-hybrid cv. Thumper). Plants were grown under ambient [CO_2] (a[CO_2], ~400 μmol mol^{-1}) or elevated [CO_2] (e[CO_2], ~700 μmol mol^{-1}) in a glasshouse. Two water treatments (well-watered and drought) were established in each [CO_2] treatment. During the growing season leaf gas exchange parameters were measured. Leaf area was measured at 80 days after sowing. Aboveground biomass, seed yield, yield components and root biomass in four different soil layers (Layer 1: 0–20 cm, Layer 2: 21–40 cm, Layer 3: 41–60 cm and Layer 4: 61–80 cm depth) were measured at maturity. Weekly water use was determined gravimetrically.

Elevated [CO_2] stimulated seed yield (38%), aboveground biomass (34%), root biomass (42%), leaf area (42%) and leaf biomass (41%). Whilst e[CO_2] stimulated root biomass in all soil layers, this stimulation was greater in the deeper than upper soil layers, and was associated with greater extraction of deeper soil water under e[CO_2]. The cultivar with greater stimulation of deeper root biomass under e[CO_2] showed greater yield benefit from the 'CO$_2$ fertilisation effect'. Under well-watered conditions, e[CO_2]-induced reductions of stomatal conductance (g_s) balanced the effect of increased leaf area on water use, resulting in similar water use compared to a[CO_2]. In contrast, under drought conditions, water use was greater under e[CO_2] than a[CO_2]. The 'CO$_2$ fertilisation effect' depended on cultivar and water treatment. Under well-watered conditions, aboveground biomass of the hybrid cultivar benefitted more from the 'CO$_2$ fertilisation effect'. However, under drought both aboveground biomass and seed yield of the non-hybrid cultivar benefitted more from the 'CO$_2$ fertilisation effect'. These findings show that interactions between environmental conditions (here experimental water treatments) and expression of genotypic traits (here differences between cultivars) play a decisive role in determining potential yield and growth benefits from rising [CO_2].

1. Introduction

Having reached a global average of ~410 μmol mol^{-1} in 2018 (NOAA, 2018), the concentration of atmospheric CO_2 ([CO_2]) continues to rise and is likely to surpass 700 μmol mol^{-1} by 2100 (IPCC, 2013). Driven by [CO_2], global temperatures are rising with consequences such as alteration of rainfall patterns and projected decreases in annual and winter growing season rainfall in some regions (Watson et al., 2017). Rainfall decreases in Mediterranean-type environments will increase the severity of late-season drought and adversely impact crop growth and yield (Farooq et al., 2017).

Rising [CO_2] will increase photosynthesis, growth and productivity of C_3 plants through the so-called 'CO$_2$ fertilisation effect' (Ainsworth and Long, 2005; Leakey et al., 2009). The magnitude of this effect is

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often assumed to be greater under drier than well-watered conditions (Leakey et al., 2009; Kimball, 2016). This is due to the well-documented reduction of stomatal conductance (g_s) under elevated [CO_2] (e[CO_2]) (Ainsworth and Long, 2005), which can lower crop water use during the early growth stages (Leakey et al., 2009; Kimball, 2016) and conserve soil water for the critical grain filling period (Burkart et al., 2011; Hussain et al., 2013). This extra soil water may extend the period during which carbon and biomass gain can be stimulated by e[CO_2] and hence mitigate the effect of drought on crop productivity (Manderscheid and Weigel, 2007).

Some recent experimental (Gray et al., 2016), meta-analytical (van der Kooi et al., 2016) and modelling (Jin et al., 2018) analyses however have not supported this assumption. The net response of water use under e[CO_2] is dependent on the balance between e[CO_2]-induced increases in leaf-level water use efficiency on the one hand, and e[CO_2]-stimulation of biomass and, especially, transpiring leaf area on the other hand (Gray et al., 2016). Greater leaf area combined with increased canopy temperature under e[CO_2] can increase total transpiration, possibly overcompensating for water savings through reduced g_s and even exacerbating drought effects (Manea and Leishman, 2014). The interactions between e[CO_2] and drought therefore warrant detailed study to better understand how drought affects the CO_2-stimulation of crop yield and how e[CO_2] affects water use.

In addition to environmental conditions, genotypic variability can also influence the impact of [CO_2] on plant growth. Inconsistencies among cultivars in their response to the 'CO_2 fertilisation effect' are associated with differences in harvest index (Bishop et al., 2015), fertile spikes/pod number (Johannessen et al., 2002; Tausz-Posch et al., 2015), seed number and/or seed size (Tausz-Posch et al., 2015), transpiration efficiency (Christy et al., 2018) and potentially rooting dynamics (Bahrami et al., 2015). For example, greater root biomass and length of vigorous hybrid cultivars than that of non-hybrid cultivars (Palta et al., 2007) combined with an enhanced capacity to use the additional carbon through stronger sink generation (more and larger seeds) led to greater response of vigorous hybrid rice cultivars to e[CO_2] compared to non-hybrid cultivars (Liu et al., 2008; Yang et al., 2009b).

An important option to mitigate drought is to improve the access to deeper soil water by extended root growth (Manschadi et al., 2006; Wasson et al., 2012). Elevated [CO_2] stimulates the root growth (Chaudhuri et al., 1990; Madhu and Hatfield, 2013; Uddin et al., 2018a), and this stimulation can be greater than that of aboveground biomass (Kimball et al., 2002). Roots of plants exposed to e[CO_2] grow faster and become more numerous, thicker and longer (Chaudhuri et al., 1990; Madhu and Hatfield, 2013). Root growth stimulation by e[CO_2] varies depending on the rooting depth and can be greater at both shallow (Chaudhuri et al., 1990; VanVuuren et al., 1997) and deeper soil layers (Burkart et al., 2004). It remains to be tested whether e[CO_2]-stimulation of root growth can improve access to deeper soil water and changes water extraction profiles.

Many [CO_2] enrichment studies used plants in relatively small pots for their experiments (for example, canola grown in small 1 L (Qaderi et al., 2006) or 25.5 cm high 7 L pots (Franzaring et al., 2011)) and while stimulation of root growth by e[CO_2] was observed, a vertical distribution of roots was not reported. Using small containers can create limitations in the rhizosphere that may alter the expression of crop responses, especially root growth to e[CO_2], which might be expected in taller containers (Bourgault et al., 2016). Taller containers allow for greater expression of root growth to different depths and may therefore allow for improved analysis of the vertical distribution of roots under e[CO_2] and its effect on soil water extraction from deeper soil layers.

To investigate the interactive response between [CO_2] and drought we selected canola (*Brassica napus* L.) due to both its high economic value and reported sensitivity to drought. Canola is an important oilseed crop and currently ranks second in global importance as a source of protein for livestock (Högy et al., 2010) and third for vegetable oil

(FAOSTAT, 2018). Earlier studies reported greater drought sensitivity of canola than other crops such as wheat (Hess et al., 2015). Depending on the growth stage, drought can reduce the seed yield of canola by up to 50% (Sinaki et al., 2007). Regardless, canola is frequently grown in Mediterranean-type environments where terminal drought regularly occurs (Maaz et al., 2018). One recent study failed to detect e[CO_2]-induced mitigation of drought effects on biomass and physiological processes of young canola seedlings (Faralli et al., 2017). It is therefore of great interest whether and, if yes, by which mechanisms and to what extent, e[CO_2] can mitigate drought effects on seed yield of canola.

To address the above issues, we conducted a glasshouse experiment growing two canola cultivars contrasting in growth traits (vigorous hybrid cv. Hyola 50 and non-hybrid cv. Thumper) in a factorial combination of ambient [CO_2] (a[CO_2], $\sim 400\,\mu mol\,mol^{-1}$) or e[$CO_2$] ($\sim 700\,\mu mol\,mol^{-1}$) with two water treatments (well-watered and drought), fully replicated over two years. We tested the following hypotheses:

(1) The 'CO_2 fertilisation effect' will be greater under well-watered than drought conditions.
(2) Water use will be greater under e[CO_2], because stimulation of transpiring leaf area overcompensates for reductions in stomatal conductance.
(3) The 'CO_2 fertilisation effect' will be greater for a vigorous hybrid cultivar (cv. Hyola 50) than for a non-hybrid cultivar (cv. Thumper).
(4) Elevated [CO_2] will stimulate root growth and this will change soil water extraction profiles.

2. Materials and methods

2.1. Experimental set-up

The experiment was conducted in a glasshouse at the Creswick Campus of The University of Melbourne, Creswick, Victoria, Australia (37°25′24.2″ S, 143°54′1.6″ E, elevation 465 m), in 2015 and replicated in 2017. Two canola (*Brassica napus* L.) cultivars of contrasting vigour and growth (vigorous hybrid cv. Hyola 50 and non-hybrid cv. Thumper) were grown in either an a[CO_2] ($\sim 400\,\mu mol\,mol^{-1}$) or an e[$CO_2$] ($\sim 700\,\mu mol\,mol^{-1}$) glasshouse chamber (glasshouse subdivision) under natural light, which was approximately 70–80% of the outside light level and was supplemented by fluorescent lamps (Wujeska-Klause et al., 2015) set to a 14/10 h day/night photoperiod and 22 ± 2.4/13 ± 1.9 °C (mean maximum/minimum temperature ± SE) temperature regimes. The supplemental CO_2 for the e[CO_2] chamber was supplied during the daytime only. To control for any unspecific chamber effect, [CO_2] treatments were swapped between chambers for the replication in the second year. Plants were randomly relocated within the respective chambers every week to avoid any position and border effects on plant responses.

2.2. Preparation of growth medium and PVC columns

Canola was grown in polyvinyl chloride (PVC) columns of 10 cm diameter and 80 cm height. The experimental soil was grey sandy loam with pH 6.8, EC 645 $\mu S\,cm^{-1}$ and field capacity (FC) for soil water of 28% (v/v; determined after three wetting–drying cycles to reach equilibrium) obtained from a local field in Ballarat, Victoria, Australia. After sieving through a 2 mm sieve, the soil was thoroughly mixed with basal nutrients at a rate of 20 mg N as urea, P as KH_2PO_4 and Mg as $MgSO_4$ as well as 10 mg Zn as $ZnSO_4$, Fe as $FeSO_4$, Cu as $CuSO_4$ and Mn as $MnSO_4$, plus 1.5 g $CaCO_3\,kg^{-1}$ of soil. About 9.8 kg oven dry soil was used to fill each column with a resulting soil density of 1.55 $Mg\,m^{-3}$. During this filling process, a plastic wire mesh (10 mm mesh) was placed at each of three different depths (20, 40 and 60 cm) to split the soil profile into four equal layers of 20 cm length (Layer 1: 1–20 cm,

Layer 2: 21–40 cm, Layer 3: 41–60 cm and Layer 4: 61–80 cm depth), facilitating clear separation at sampling. In the middle of each layer (at 10, 30, 50 and 70 cm depth) four holes of 4.5 mm diameter were drilled through the side of the PVC column to allow periodic measurement of soil water content (SWC). Ten uniformly sized seeds of canola were sown at 1.5 cm depth in each column on 5 June and 30 May in 2015 and 2017, respectively. Ten days after sowing, seedlings were thinned to the most vigorous single plant per column.

2.3. Water treatments (WT)

The columns were hand watered and SWC was maintained close to FC until 40 days after sowing. Thereafter, columns were assigned to two groups, well-watered (WW) and drought (DD) and SWC was maintained at 90 and 50% of FC, respectively by weighing each column every week and replacing the amount of water lost through evapotranspiration. To avoid excessive fluctuations of SWC, half of the amount of water evapotranspired in the previous week was added mid-week. The amount of irrigation water was recorded to estimate the weekly water use by weighing the PVC column, and weekly water uses were summed to calculate total water use during the whole growing season. Because total plant biomass at final harvest was less than 0.35% of the total column weight (PVC column + soil + soil water + plant biomass), the change in biomass was ignored in determining the water regimes (Wu et al., 2004). During the 2017 replication of the experiment, volumetric SWC of each layer was measured weekly (before irrigation) by inserting a time domain refractometer (TDR, Theta probe ML3, Delta-T Devices Ltd., Burwell, Cambridge, UK) through the pre-drilled horizontal holes in the middle of each layer. The factory default calibration was used to convert the voltage output from the TDR into SWC (v/v%).

2.4. Gas exchange measurements

Stomatal conductance (g_s), net photosynthetic assimilation rate (A_{net}), transpiration rate (E) and internal [CO_2] of one randomly selected fully expanded young leaf at mid-plant height were measured weekly from 78 to 120 days after sowing. An open-path infrared gas analyser (IRGA, Li-6400, Li-Cor, Lincoln, NE, USA) with a standard leaf chamber (clear-top with a maximum leaf area of 2×3 cm) was used to measure instantaneous gas exchange for four replicate columns in each treatment combination. The cuvette air flow rate was set to 500 μmol s^{-1} with either 400 or 700 μmol mol^{-1} of [CO_2] depending on the growth conditions. All measurements took place on clear sunny days between 9.30 to 11.30 a.m. The photosynthetically active radiation at the leaf level ranged from 600 to 850 μmol $m^{-2} s^{-1}$. Measurements were recorded after first stabilisation (generally after 90 s) and three measurements were recorded at a 5 s interval and averaged. This allowed conditions in the cuvette to reach a steady state, but did not allow stomata to adjust to the cuvette conditions. Across all measurements leaf temperatures were between 23.4 to 28.6 °C and leaf-to-air vapour pressure deficits were 0.8 to 2.7 kPa; and neither of these were different between samples and treatments on each measurement date. Intrinsic water use efficiency (iWUE) was calculated as A_{net} divided by g_s.

2.5. Growth and yield parameters

Eighty days after sowing (40 days after water treatment started) total leaf area was measured using a leaf area meter (LI-3100C Area Meter, Licor, Lincoln, NE, USA). Senescent leaves from individual plants were collected every four to five days and stored separately to estimate total leaf production until final harvest. Final harvesting of e[CO_2] and a[CO_2] grown canola were done on 7 and 19 November in 2015 and on 2 and 15 November in 2017, respectively. At harvest, siliqua number $plant^{-1}$ were recorded. Twenty fully matured siliquae were randomly selected from each plant to determine seed

characteristics. Seeds were removed from each siliqua by threshing, separated into sound (fully developed) and aborted (not developed, shrinkage) categories, counted, and weighed. After threshing, seeds were dried to constant weight at room temperature (Franzaring et al., 2011). All other individual plant parts were oven dried at 70 °C for 72 h and their dry biomass was recorded separately. Harvest index (HI) was calculated as seed yield divided by aboveground biomass. Individual sound seed weight was calculated by dividing the sound seed weight of 20 siliquae by their respective sound seed number. Immediately after harvest, columns were disassembled and separated into four layers. There was no sign of roots curling up at the bottom of the columns, indicating a sufficient length of the column for un-restricted longitudinal root growth. The roots in each layer were collected from the soil by washing with tap water and sieving with a 2 mm sieve. Roots were oven dried at 70 °C for 72 h and their dry biomass recorded. Total root biomass was calculated as the sum of root biomass across all four layers. Water use efficiency of biomass (WUEb) and seed yield (WUEy) were calculated by dividing the biomass and seed yield with total water use.

2.6. Statistical analysis

The experimental design was a nested split-plot design with 2 [CO_2] (main-plots), 2 cultivars and 2 water treatments nested within the CO_2 with 4 replications for a total of $2 \times 2 \times 2 \times 4 = 32$ experimental columns in each of the two years. The effects of CO_2, cultivars, and water treatments were considered fixed effects and year and replicates as random effects in a linear mixed effects model analysed with R package 'nlme' (Pinheiro et al., 2018). To explicitly investigate the role of soil depth (layer) on root biomass and SWC, these two parameters (ln transformed for root biomass) were further analysed including layer as an additional fixed effect and the respective column ID as a random effect. Repeatedly measured parameters (leaf gas exchanges and SWC) are reported for one year, hence the sample size is n = 4 (n = 8 for all other reported parameters) and for them days after sowing instead of year was used as a random effect. Homogeneity of variances was evaluated visually, and the model was adjusted when stepwise evaluation of the model indicated an improvement after correction. Linear regression analyses (R package "stats") were used to assess the relationship between water use and measured parameters. Data analysis was performed in R version 3.4.3 (R Core Team, 2017).

3. Results

3.1. Soil water

Soil water content (measured during the 2017 season before each irrigation, therefore representing the minima reached in the rewatering cycles) was consistently lower under e[CO_2] than a[CO_2] in all soil layers (Fig. 1). Averaged across all soil layers and measurement dates, SWC in the DD treatment was 26% lower than under WW. In layers 2 and 4, SWC was lower for cv. Thumper than for cv. Hyola 50. In layer 3, e[CO_2]-induced depletion of soil water by cv. Hyola 50 was greater (8%) under WW than DD conditions (2%). In the same layer cv. Thumper grown under e[CO_2] depleted soil water more under DD (9%) than under WW (4%) conditions. In the deepest layer (layer 4), e[CO_2]-induced depletion of soil water was greater for cv. Thumper (11%) than cv. Hyola 50 (6%).

3.2. Gas exchange parameters

Elevated [CO_2] stimulated A_{net} of canola more under WW (25%) than under DD (19%) conditions (Fig. 2A). The cv. Thumper had 18% greater A_{net} than cv. Hyola 50. Elevated [CO_2] reduced g_s, and this reduction was greater under WW (18%) than DD (11%) conditions (Fig. 2B). Increased A_{net} and decreased g_s resulted in 43% greater iWUE

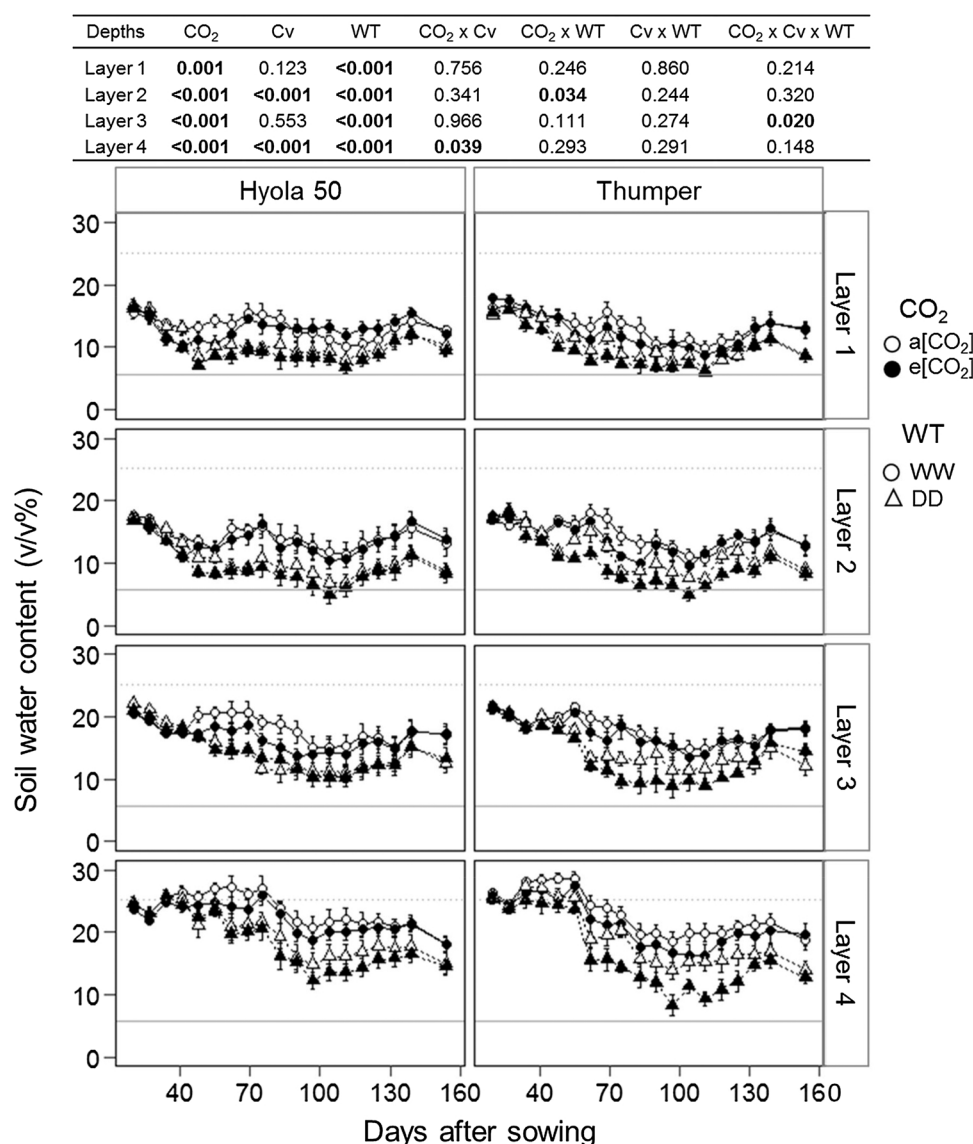| Depths	CO_2	Cv	WT	CO_2 x Cv	CO_2 x WT	Cv x WT	CO_2 x Cv x WT
Layer 1	**0.001**	0.123	**<0.001**	0.756	0.246	0.860	0.214
Layer 2	**<0.001**	**<0.001**	**<0.001**	0.341	**0.034**	0.244	0.320
Layer 3	**<0.001**	0.553	**<0.001**	0.966	0.111	0.274	**0.020**
Layer 4	**<0.001**	**<0.001**	**<0.001**	**0.039**	0.293	0.291	0.148

Fig. 1. Soil water content measured weekly in the middle of four soil layers (Layer 1: 0–20 cm, Layer 2: 21–40 cm, Layer 3: 41–60 cm and Layer 4: 61–80 cm depth) of the PVC column used for growing canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper under ambient $[CO_2]$ (a$[CO_2]$, ~400 µmol mol^{-1}) or elevated $[CO_2]$ (e$[CO_2]$, ~700 µmol mol^{-1}) with contrasting water treatments (well-watered: WW or drought: DD) in 2017. Each data point represents the mean values (standard errors) of n = 4 replicates. Soil water content at 90% of field capacity (dotted line) and at permanent wilting point (adapted from Veihmeyer (1956); continuous line) are indicated by horizontal lines. P values of mixed effects model with CO_2, cultivar (Cv) and water treatments (WT) are presented in the above table. Statistically significant effects are shown in bold numbers.

for canola grown under e$[CO_2]$ than under a$[CO_2]$ (Table 1). This e$[CO_2]$-stimulation of iWUE was greater for cv. Thumper than cv. Hyola 50 and WW than DD conditions. Elevated $[CO_2]$ reduced E under WW (16%) but left it unchanged under DD conditions (Fig. 2C). Transpiration rate of cv. Thumper was greater than that of cv. Hyola 50. Intercellular $[CO_2]$ was greater under e$[CO_2]$ than a$[CO_2]$, cv. Thumper than cv. Hyola 50 and WW than DD conditions (Table 1).

3.3. Leaf area and leaf biomass

Canola grown under e$[CO_2]$ had greater leaf area (42%) and leaf biomass (41%) than under a$[CO_2]$ (Fig. 2D; Table 1). Elevated $[CO_2]$-stimulation of leaf area was greater for cv. Thumper (54%) than cv. Hyola 50 (30%) and greater under WW than DD conditions. Although cv. Thumper had 17% larger leaf area than cv. Hyola 50, the difference in leaf biomass was only 10% and not statistically significant. Drought significantly reduced leaf area by 41% and leaf biomass by 72%. Total water use was positively correlated with both, leaf biomass (Fig. 3A) and leaf area (Fig. 3B).

3.4. Root biomass

Elevated $[CO_2]$ increased the root growth of canola in all four soil

layers, with total root biomass 42% greater than under a$[CO_2]$ (Fig. 4). The effect of e$[CO_2]$ on root biomass however was inconsistent across cultivars and soil layers (CO_2 x Cv x Layer; P = 0.019). The greatest stimulation of root growth by e$[CO_2]$ was observed in the deepest layer (layer 4; 286%), and the lowest response in layer 2 (21%). Elevated $[CO_2]$ stimulated root growth in layers 1 and 3 by 40 and 69%, respectively (CO_2 x Layer; P < 0.001). Similar to e$[CO_2]$, WW conditions increased the total root biomass (37%) relative to DD by increasing the root growth in all layers. However, in contrast with the e$[CO_2]$-effect, stimulation of root growth under WW relative to DD conditions was greatest in layer 2 (67%) and similar (~36%) in other layers (WT x Layer; P = 0.008).

The effect of cultivar on root growth varied in different layers (Cv x Layer; P < 0.001). In the top soil layer (layer 1), root biomass of cv. Hyola 50 was greater (10%) than that of cv. Thumper. In contrast, root biomass of cv. Thumper was 62 and 81% greater than cv. Hyola 50 in layers 3 and 4 respectively. Since the greatest proportion of total root biomass was in layer 1, greater root biomass of cv. Hyola 50 at that layer made up for greater root biomass of cv. Thumper in other layers so that the cultivars showed no significant difference in total root biomass. Irrespective of cultivars and water treatments, total water use was positively correlated with total root biomass (Fig. 3C).
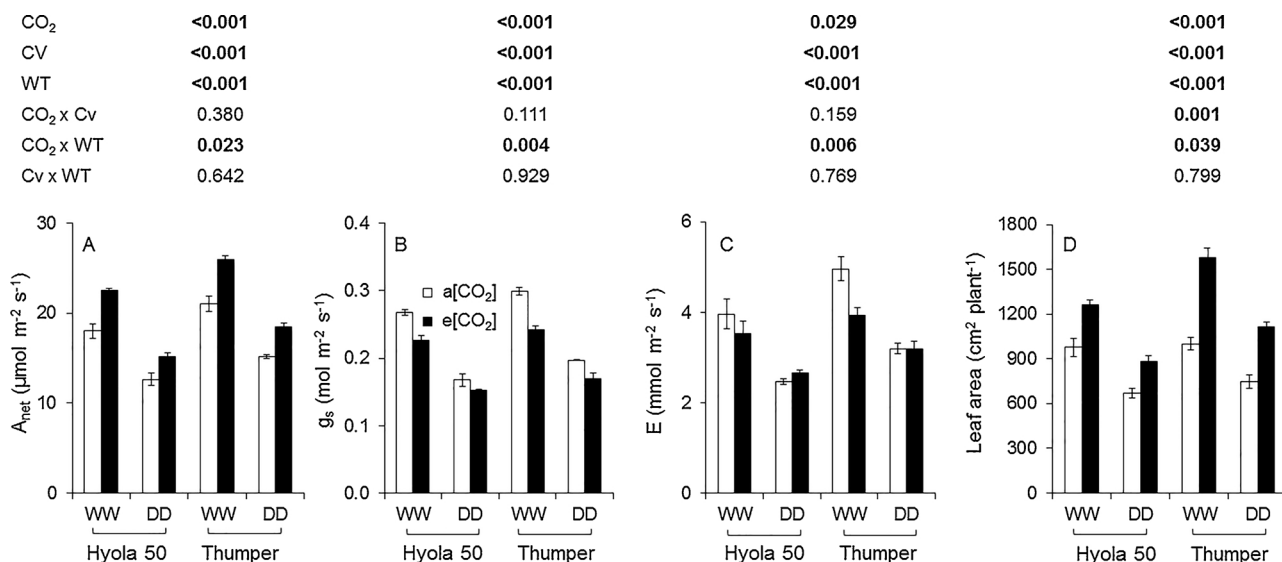
CO₂	**<0.001**	**<0.001**	**0.029**	**<0.001**
CV	**<0.001**	**<0.001**	**<0.001**	**<0.001**
WT	**<0.001**	**<0.001**	**<0.001**	**<0.001**
CO₂ x Cv	0.380	0.111	0.159	**0.001**
CO₂ x WT	**0.023**	**0.004**	**0.006**	**0.039**
Cv x WT	0.642	0.929	0.769	0.799

Fig. 2. (A) Net photosynthetic assimilation rate (A_{net}), (B) stomatal conductance (g_s), (C) transpiration rate (E) and (D) leaf area (measured at 80 days after sowing) of canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper under ambient [CO_2] (a[CO_2], ~ 400 µmol mol⁻¹) or elevated [CO_2] (e[CO_2], ~ 700 µmol mol⁻¹) with contrasting water treatments (well-watered: WW or drought: DD). Each bar represents the mean (standard errors). For leaf area n = 8 replicates (measured in 2015 and 2017), for gas exchange parameters n = 4, each replicate is averaged of 7 measurements made in 2017. P values of mixed effects model with CO_2, cultivar (Cv) and water treatments (WT) are presented above each graph. Statistically significant effects are shown in bold numbers. No statistically significant three-way interactions were observed for any of the parameters (all P ≥ 0.10).

3.5. Growth and yield parameters

Elevated [CO_2]-stimulation of siliqua number was greater for cv. Hyola 50 (51%) than cv. Thumper (31%; Table 1). Well-watered plants produced 38% more siliqua than DD ones, and the increase of siliqua number under WW relative to DD conditions was double for cv. Hyola 50 (50%) than cv. Thumper (25%).

Elevated [CO_2] increased the number of sound seeds siliqua⁻¹ of cv. Thumper by 13%, but not for cv. Hyola 50 (~1%). Elevated [CO_2] also reduced the number of aborted seeds siliqua⁻¹ compared to a[CO_2], whereby cv. Thumper showed a greater reduction (46%) than cv. Hyola 50 (7%). Individual sound seed weight of cv. Thumper was 15% greater than that of cv. Hyola 50. Elevated [CO_2] increased the sound seed weight of cv. Thumper (4%) but decreased it for cv. Hyola 50 (12%; Table 1).

A higher number of siliqua plant⁻¹ and sound seeds siliqua⁻¹ along with larger individual sound seeds resulted in 38% greater seed yield for canola grown under e[CO_2] than under a[CO_2] (Table 1). Elevated [CO_2]-stimulation of seed yield was greater for cv. Thumper (50%) than for cv. Hyola 50 (25%). Drought-induced reduction of seed yield of cv. Hyola 50 (67%) was greater than for cv. Thumper (29%).

Canola grown under e[CO_2] had on average 34% greater aboveground biomass than grown under a[CO_2] with cv. Thumper showing a greater e[CO_2]-stimulation under DD (45%) than WW (18%) conditions (Table 1). In contrast, e[CO_2]-stimulation of aboveground biomass of cv. Hyola 50 was somewhat greater under WW (42%) than DD conditions (37%). Stem and chaff biomass showed a similar trend as aboveground biomass (Table 1). Plants grown under e[CO_2] were taller, had a larger leaf area, more siliquae and greater numbers of seeds, which resulted in greater aboveground biomass under e[CO_2] than a [CO_2] (Table 1). Elevated [CO_2] reduced the harvest index (HI) of cv. Hyola 50 by 23% under WW conditions, but increased it by 7% under DD conditions. Irrespective of water treatments, e[CO_2] increased the HI of cv. Thumper, but to a greater extent under WW (21%) than DD conditions (14%, Table 1).

3.6. Water use and water use efficiencies

During the entire growing season cv. Thumper used 10% more water than cv. Hyola 50 (Table 1). Compared to a[CO_2], canola treated with e[CO_2] used more (12%) water under DD conditions, but this difference was not apparent in well-watered conditions (Table 1; significant CO_2 x WT interaction). Since the e[CO_2]-stimulation of aboveground biomass was greater than the increase in water consumption (Fig. 5A), WUEb of canola increased by 30% under e[CO_2] (Table 1). The WUEy increased even more (35%) under e[CO_2] (Fig. 5B, Table 1), due to the additional stimulation of HI by e[CO_2]. Imposition of drought reduced the WUEb of cv. Hyola 50 more (18%) than that of cv. Thumper (2%). Under drought conditions, the difference between the two cultivars was even greater for WUEy, where drought reduced the WUEy of cv. Hyola 50 (16%) but increased it for cv. Thumper (14%; Table 1).

4. Discussion

The drought treatment in our current study caused detectable reductions in g_s, but seasonally averaged g_s remained at or above 0.15 mol m⁻² s⁻¹, indicating only mild water stress (Flexas and Medrano, 2002) that might have limited photosynthesis through restricted CO_2-diffusion, but was unlikely to have caused any physiological damage (Flexas et al., 2006). On the other hand, SWC were near the lower threshold of plant available water, which would indicate some measure of drought stress. As SWC were measured immediately before rewatering, the values represent the minima reached during the rewatering cycle, whereas gas exchange measurements were taken between watering events (normally one day before a fraction of water was added mid-week) when these minima were not yet reached. Both measures together indicate that drought treated plants experienced mild to moderate drought stress, restricting gas exchange and growth, but were unlikely to have suffered severe stress with associated direct physiological damage (Flexas et al., 2006).

Table 1

Growth and yield parameters of canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper grown under ambient [CO$_2$] (a[CO$_2$], ~400 μmol mol^{-1}) or elevated [CO$_2$] (e[CO$_2$], ~700 μmol mol^{-1}) with contrasting water treatments (well-watered: WW or drought: DD). Mean values and standard errors of n = 8 replicates (4 replicates in each two experiments run in 2015 and 2017; for siliqua parameters 20 siliquae averaged for each replicate) except for iWUE and intercellular [CO$_2$] (n = 4; each averaged from 7 repeated measurements). P values of mixed effects model with CO$_2$, cultivar (Cv) and water treatments (WT) as well as their interactions are presented here. Statistically significant (P < 0.1) effects are shown in bold numbers. iWUE intrinsic water use efficiency; WUEb water use efficiency for biomass; WUEy water use efficiency for seed yield.

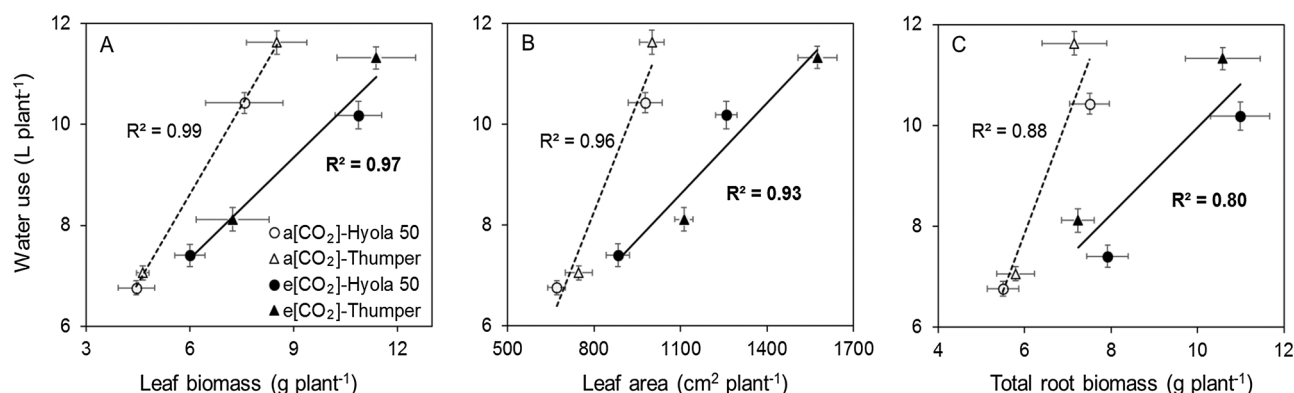| Response parameters	Hyola 50				Thumper				P value						
	WW		DD		WW		DD		CO$_2$	Cv	WT	CO$_2$×Cv	CO$_2$×WT	Cv×WT	CO$_2$×Cv×WT
	a[CO$_2$]	e[CO$_2$]	a[CO$_2$]	e[CO$_2$]	a[CO$_2$]	e[CO$_2$]	a[CO$_2$]	e[CO$_2$]							
iWUE (μmol mol^{-1})	67.3 ± 1.8	99.7 ± 3.7	75.5 ± 1.5	98.7 ± 2.2	70.2 ± 2.2	107.5 ± 2.2	76.8 ± 1.1	109.0 ± 3.6	**< 0.001**	**0.003**	**0.034**	**0.050**	**0.044**	0.896	0.554
Intercellular [CO$_2$] (μmol mol^{-1})	230.3 ± 14	574.3 ± 12	161.5 ± 22	421.7 ± 59	263.6 ± 9	610.1 ± 9	222.8 ± 19	529.3 ± 24	**< 0.001**	**0.001**	**< 0.001**	0.462	**0.070**	0.139	0.507
Siliquae (# plant^{-1})	128.9 ± 7.1	185.8 ± 5.2	80.3 ± 2.4	129.5 ± 6.1	101.0 ± 6.5	131.4 ± 11.1	80.5 ± 3.0	106.1 ± 2.3	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.457	**< 0.001**	0.863
Sound seeds (# siliqua^{-1})	16.7 ± 0.2	16.0 ± 0.7	14.1 ± 0.8	15.2 ± 0.5	18.6 ± 0.5	20.8 ± 0.5	16.2 ± 0.8	18.7 ± 0.4	**0.003**	**< 0.001**	**< 0.001**	**0.012**	0.205	0.503	0.348
Aborted seeds (# siliqua^{-1})	0.83 ± 0.11	0.86 ± 0.07	1.2 ± 0.1	1.0 ± 0.1	0.73 ± 0.09	0.38 ± 0.05	0.73 ± 0.05	0.41 ± 0.05	**0.005**	**< 0.001**	**0.038**	**0.026**	0.529	**0.063**	0.389
Leaf biomass (g plant^{-1})	7.6 ± 1.1	10.9 ± 0.7	4.5 ± 0.5	6.0 ± 0.4	8.5 ± 0.9	11.4 ± 1.1	4.6 ± 0.2	7.2 ± 1.1	**< 0.001**	0.183	**< 0.001**	0.569	0.388	0.991	0.526
Stem biomass (g plant^{-1})	11.0 ± 0.5	15.5 ± 1.0	6.8 ± 0.5	8.0 ± 0.6	10.1 ± 1.0	10.8 ± 1.1	5.9 ± 0.6	7.7 ± 0.8	**< 0.001**	**< 0.001**	**< 0.001**	**0.069**	0.189	**0.015**	**0.012**
Siliquae biomass (g plant^{-1})	12.1 ± 0.7	17.2 ± 1.6	7.0 ± 0.4	11.1 ± 1.0	14.6 ± 0.9	17.1 ± 1.7	8.9 ± 0.5	13.2 ± 0.5	**< 0.001**	**< 0.001**	**< 0.001**	0.797	0.731	0.546	0.252
Chaffs biomass (g plant^{-1})	5.5 ± 0.5	9.8 ± 1.2	3.7 ± 0.3	6.1 ± 0.9	8.4 ± 0.7	8.4 ± 1.2	4.5 ± 0.3	6.1 ± 0.6	**< 0.001**	0.233	**< 0.001**	**0.011**	0.877	0.696	**0.097**
Sound seed weight (mg)	3.9 ± 0.14	3.4 ± 0.13	3.9 ± 0.04	3.4 ± 0.07	4.1 ± 0.06	4.3 ± 0.27	4.1 ± 0.13	4.3 ± 0.06	**0.023**	**< 0.001**	0.734	**< 0.001**	0.946	0.986	0.745
Seed yield (g plant^{-1})	6.6 ± 0.5	7.4 ± 0.7	3.4 ± 0.2	5.0 ± 0.3	6.2 ± 0.6	8.7 ± 0.8	4.4 ± 0.2	7.2 ± 0.4	**< 0.001**	**0.002**	**< 0.001**	**0.028**	0.354	**0.073**	0.722
Aboveground biomass (g plant^{-1})	30.6 ± 1.9	43.5 ± 2.5	18.3 ± 0.7	25.1 ± 1.1	33.2 ± 2.6	39.3 ± 3.8	19.4 ± 1.2	28.2 ± 1.9	**< 0.001**	0.617	**< 0.001**	0.340	0.507	0.264	**0.091**
Harvest index	0.22 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.23 ± 0.01	0.26 ± 0.01	0.289	**< 0.001**	**0.041**	**0.002**	**0.089**	**0.022**	**0.042**
Water use (L plant^{-1})	10.43 ± 0.2	10.2 ± 0.3	6.76 ± 0.1	7.4 ± 0.2	11.63 ± 0.2	11.32 ± 0.2	7.06 ± 0.1	8.12 ± 0.2	**0.041**	**< 0.001**	**< 0.001**	0.526	**< 0.001**	**0.02**	0.393
WUEb (g L^{-1})	2.9 ± 0.2	4.3 ± 0.3	2.7 ± 0.1	3.4 ± 0.1	2.9 ± 0.2	3.5 ± 0.3	2.7 ± 0.1	3.5 ± 0.3	**< 0.001**	0.191	**0.004**	0.231	0.122	**0.068**	0.13
WUEy (g L^{-1})	0.63 ± 0.04	0.73 ± 0.07	0.50 ± 0.03	0.67 ± 0.03	0.54 ± 0.06	0.77 ± 0.08	0.62 ± 0.03	0.90 ± 0.07	**< 0.001**	**0.001**	0.887	**0.092**	0.41	**0.007**	0.882

Fig. 3. Linear regressions between total water use and leaf biomass (A), leaf area (B) and total root biomass (C) of canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper grown under ambient [CO_2] (a[CO_2], ~ 400 µmol mol^{-1}; broken line; R^2 in normal font) or elevated [CO_2] (e[CO_2], ~ 700 µmol mol^{-1}, continuous line; R^2 in bold font) across two water treatments (well-watered: WW or drought: DD). All the observed relationships were statistically significant (P < 0.001). Each data point represents the mean (standard errors) of n = 8 replicates.

Fig. 4. Root biomass in four soil layers (Layer 1: 0–20 cm, Layer 2: 21–40 cm, Layer 3: 41–60 cm and Layer 4: 61–80 cm depth) and total root biomass (0–80 cm: sum of four layers) of canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper grown under ambient [CO_2] (a[CO_2], ~ 400 µmol mol^{-1}) or elevated [CO_2] (e[CO_2], ~ 700 µmol mol^{-1}) with contrasting water treatments (well-watered: WW or drought: DD) in 2015 and 2017. Each bar represents the mean (standard errors) of n = 8 replicates. P values of mixed effects model with CO_2, cultivar (Cv) and water treatments (WT) are presented above each graph. Statistically significant effects are shown in bold numbers. No statistically significant three-way interactions were observed for root biomass in any of the soil layers (all P ≥ 0.10).

4.1. The effect of water supply on 'CO$_2$ fertilisation effect' of canola was cultivar specific

Our first hypothesis was that the 'CO$_2$ fertilisation effect' is greater under well-watered than drought conditions. Consistent with the trend observed for canola in earlier [CO_2] enrichment studies, we found stimulation of photosynthesis, growth and seed yield under e[CO_2], and yield increases were due to increased siliqua and seed numbers per
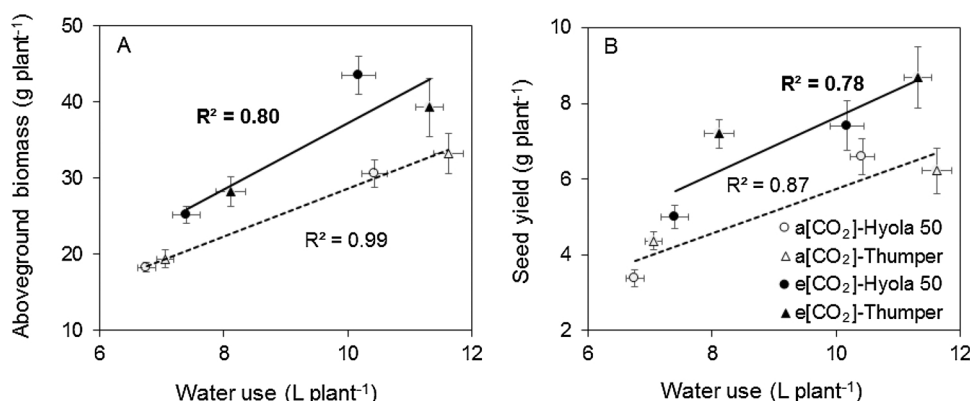

Fig. 5. Linear regressions between total water use and aboveground biomass (A) and seed yield (B) of canola (*Brassica napus* L.) cv. Hyola 50 and cv. Thumper grown under ambient [CO_2] (a[CO_2], ~ 400 µmol mol^{-1}; broken line; R^2 in normal font) or elevated [CO_2] (e[CO_2], ~ 700 µmol mol^{-1}, continuous line; R^2 in bold font) across two water treatments (well-watered: WW or drought: DD). All the observed relationships were statistically significant (P < 0.001). Each data point represents the mean (standard errors) of n = 8 replicates.

plant as well as larger seed size (Johannessen et al., 2002; Qaderi and Reid, 2005; Frenck et al., 2011). Even though CO_2-stimulation of photosynthesis was greater under well-watered conditions, there were no significant CO_2 x WT interactions for yield or biomass, hence there is no general evidence to support the hypothesis.

There were however complex interactions with water treatment, water use and cultivar characteristics, discussed in detail in regards to the following hypotheses. One example is the association of stimulation in seed yield and an increase in HI, i. e. greater seed production relative to biomass (Aranjuelo et al., 2013). This was also observed in our study, but the effect of e[CO_2] on HI was dependent on cultivar and water treatment, due to the inconsistent increases of aboveground biomass by e[CO_2] across cultivar and water treatments. Whilst greater stimulation of aboveground biomass by e[CO_2] was previously reported for hybrid than non-hybrid cultivars (Laza et al., 2001; Yang et al., 2009a), in our study this trend was only found under well-watered conditions (mostly due to stimulation of stem and chaff biomass; Table 1). In contrast, under drought conditions, e[CO_2]-stimulation of both aboveground biomass and seed yield were greater for the non-hybrid cultivar, underlining the importance of genotype x environment interactions in CO_2-responsiveness. With respect to our first hypothesis, the answer has to be modified depending on cultivar, whereby the capacity to use additional photosynthates for seed production may determine the direction of the CO_2 x WT interaction.

4.2. The effect of e[CO_2] on water use depends on water treatment

Our second hypothesis was that water use will be greater under e[CO_2]. One of the major drivers of crop water use is canopy transpiration, which depends on the combination of g_s and leaf area (Samarakoon et al., 1995). Confirming this, our results showed significant positive correlations of leaf biomass and leaf area with water use, across both cultivars and water treatments. The increased leaf area under e[CO_2] noted in our study is in line with trends generally observed for canola (Qaderi and Reid, 2005; Faralli et al., 2017), grasses (Manea and Leishman, 2014) and other crops (Bunce, 2016) from earlier [CO_2] enrichment studies. However, the e[CO_2]-stimulation of leaf area was dependent on cultivar and water treatments, and the effect of e[CO_2] on g_s depended on water treatment, too, so that confirmation of our second hypothesis depended on the water treatment:

(1) For well-watered plants, overall leaf area changes combined with greater reduction in g_s resulted in no apparent change in water use under e[CO_2] compared to a[CO_2], which is in line with some earlier studies (Kimball et al., 1999; Hunsaker et al., 2000). Soil water content data recorded in the second season suggest that e[CO_2]-grown plants depleted more water than a[CO_2] grown ones, and, based on these data, would have used more water in a watering regime with a fixed rewatering target. As gravimetric water use and volumetric soil water measurements have different measurement errors, SWC was only measured in one season, and weekly SWC data represent one particular (minimum) point in the watering cycle, the apparent discrepancy is, therefore, not too surprising. Furthermore, due to accelerated maturity under e[CO_2], a[CO_2]-grown plants were harvested later and received additional water increasing their total seasonal water use. But most importantly, neither the gravimetric method for total water use, nor the SWC measurements indicated water savings under e[CO_2].

(2) Under drought conditions, smaller reduction of g_s combined with stimulated leaf area resulted in greater total water use under e[CO_2] than a[CO_2]. If drought causes stomata to close much of the time, then e[CO_2] may have very little or no effect on g_s (Wall et al., 2001, 2011) and therefore seasonal water use (Kimball, 2016). Greater water use by rising [CO_2] was previously reported from some e[CO_2] experiments (Wu and Wang, 2000; Wu et al., 2004). This increased water use also resulted in greater soil water

depletion under e[CO_2], and therefore reduced the SWC at different soil layers as measured in the second season. This finding agrees with some earlier studies under drought conditions, where there was no soil water conservation effect of e[CO_2] (Manea and Leishman, 2014; Gray et al., 2016).

Small or no change in water use coupled with the increased aboveground biomass and seed yield under e[CO_2] resulted in greater WUE (Hunsaker et al., 2000). Even where water use was greater, e[CO_2] stimulated both WUEb and WUEy. Increased water use efficiency at the leaf-level resulting from the enhancement of A_{net} and reduction of g_s under e[CO_2] (Allen et al., 2011) was supported by greater stimulation of aboveground biomass and seed yield than of water use to increase WUEb and WUEy under e[CO_2]. Similar results of greater water use combined with greater WUE under e[CO_2] were previously reported for broad bean (Wu and Wang, 2000) and wheat (Wu et al., 2004). Increases in water use efficiencies under e[CO_2] indicate that higher [CO_2] created an environment with more efficient use of water under both well-watered and drought conditions.

The experimental watering regime applied here, with regular rewatering to a defined water content, albeit well below field capacity, may have helped the plants to benefit from the 'CO_2 fertilisation effect' even during the drought treatment: Immediately after the regular rewatering events plants may have been able to operate with temporarily greater g_s and therefore greater A_{net} stimulation. In the field however, where rainfall is predicted to decrease in future climates (Watson et al., 2017), this e[CO_2]-stimulation of soil water depletion might increase the vulnerability of plants to drought (Manea and Leishman, 2014).

4.3. Elevated [CO_2] stimulated the aboveground biomass production of vigorous hybrid cultivar (Hyola 50) but seed yield of non-hybrid cultivar (Thumper)

In our third hypothesis we suggested that the 'CO_2 fertilisation effect' will be greater for the vigorous hybrid cultivar Hyola 50 than the non-hybrid cultivar Thumper. We observed genotypic variability between the cultivars in their response to e[CO_2] for aboveground biomass, yield components and seed yield. Elevated [CO_2] stimulated the aboveground biomass and seed yield of both cultivars, but this stimulation was potentially caused by different mechanisms. For the vigorous hybrid cultivar, increased seed yield under e[CO_2] was exclusively linked to an increased number of siliquae, but not to changes in the number of sound seeds per siliqua, and grain size even decreased for this cultivar. These results support the notion that the capacity to produce more siliquae is an important factor in canola seed yield response to e[CO_2] (Qaderi and Reid, 2005; Frenck et al., 2011). In contrast, seed yield stimulation by e[CO_2] in the non-hybrid cultivar was also associated with increased sound seed number, larger seeds and reduced number of aborted seeds. Earlier reports on the effect of e[CO_2] on canola seed size were contradictory, perhaps due to differences in their growing conditions (Johannessen et al., 2002; Qaderi and Reid, 2005; Franzaring et al., 2008, 2011; Ruhil et al., 2015). Our results suggest that both genetic differences between cultivars and growing conditions may play a role in such inconsistencies. Although e[CO_2] increased A_{net} of both cultivars, it increased seed size only in non-hybrid cultivar, which suggests that increased assimilate supply by e[CO_2] in itself does not increase seed size (Tausz-Posch et al., 2015).

In agreement with our hypothesis, average stimulation of aboveground biomass under e[CO_2] was greater for the vigorous hybrid cultivar (despite complex significant interactions among CO_2 x Cv x WT, average stimulation was greater for the hybrid than the non-hybrid cultivar). However, our results contradict our hypothesis for seed yield, because seed yield stimulation was greater for the non-hybrid cultivar. This contrasts with earlier studies reporting greater responsiveness of hybrid rice to e[CO_2] (Liu et al., 2008; Yang et al., 2009b). But more recent studies have reported similar or even greater response of non-

hybrid cultivars to e[CO_2] over hybrid cereal cultivars (Benlloch-Gonzalez et al., 2014b; Zhu et al., 2015). The greater stimulation of seed yield for a non-hybrid cultivar may be explained by source-sink relationships during vegetative and reproductive stages, which is an important trait that might impact the response of crops to e[CO_2] (Tausz et al., 2013). Greater availability of assimilates during the grain filling period reduces the early abortion of seeds (Miralles and Slafer, 2007) and/or increases the seed number and size (Ruhil et al., 2015) improving the fertility of developing pods/spikes (Fischer, 2007). Maximum photosynthetic production under e[CO_2] can only be achieved when superior vegetative growth is matched with high sink capacity (Tausz et al., 2013). In the current study, greater stimulation of leaf area and A_{net} under e[CO_2] in association with better access to deeper soil water (due to greater stimulation of root growth in deeper layers; see 4.4) probably improved assimilate supply of the non-hybrid cultivar. Because this greater assimilate supply was matched with a more flexible sink capacity in the non-hybrid cultivar this resulted in greater seed yield stimulation than for the hybrid cultivar (increased seed numbers and size, not only increased siliquae numbers, which are determined earlier). Cultivars lacking the capability to increase sink capacity during grain filling may fail to efficiently utilise the greater assimilate supply under e[CO_2] (Tausz-Posch et al., 2015).

4.4. Elevated [CO_2] enhanced root growth and soil water depletion from the deeper soil layers

Our fourth hypothesis was confirmed, as e[CO_2] stimulated root growth, but this stimulation was not uniform throughout the soil profile and therefore changed water extraction patterns. Stimulation of root growth by e[CO_2] is in line with the trends generally observed for canola (Qaderi et al., 2006; Franzaring et al., 2011) and other crops (Madhu and Hatfield, 2013; Uddin et al., 2018a). Elevated [CO_2] not only increases root biomass and/or length, but also changes the vertical distribution, often by stimulating root growth to a greater extent in the upper soil layers than in the deeper layers of the soil profile (Chaudhuri et al., 1990; VanVuuren et al., 1997). Our study showed e[CO_2]-induced stimulation of root growth in all four soil layers. However, in contrast to the above-mentioned studies but consistent with Burkart et al. (2004) this stimulation of root growth was greater in deeper than upper soil layers.

The magnitude of the response of root growth under e[CO_2] depends on cultivar (Benlloch-Gonzalez et al., 2014a) and/or growing conditions (Chaudhuri et al., 1990; Uddin et al., 2018a). In contrast with some earlier studies, where vigorous cultivars were reported to have deeper and bigger root systems (Liao et al., 2004; Palta et al., 2007), we did not observe significant differences between a vigorous hybrid and a non-hybrid cultivar in their total root biomass under e[CO_2]. However, the effect of e[CO_2] on root biomass was dependent on the soil layer and cultivar, with the non-hybrid cultivar showing a greater response to e[CO_2] in the two lower soil layers over the hybrid one. The vigour trait in the hybrid cultivar is perhaps not very strong, but in support of our results, a study with experimental wheat lines expressing strong early vigour traits reported even a suppression of root growth by e[CO_2] in vigorous lines (Benlloch-Gonzalez et al., 2014a).

The vertical distribution of roots and its role in accessing deeper soil water is a key trait for improving adaptation of crops in dryland Mediterranean regions (Manschadi et al., 2006). Elevated [CO_2]-stimulation of root growth in deeper soil layers can make significant contributions in maintaining physiological processes (Gallardo et al., 1994; Uddin et al., 2018b), because deeper roots of crops are younger and more efficient in extracting and supplying water compared to roots in upper layers (Gregory et al., 1978). Moreover, later in the season (during grain filling) a larger portion of plant roots die in upper soil layers compared to deeper layers (Pritchard et al., 2006). The e[CO_2]-stimulation of soil water depletion in deeper soil layers by the non-hybrid cultivar appears related to the greater e[CO_2]-stimulation of

deeper root growth for that cultivar. In water-limited agro-ecosystems, water captured by roots in deeper soil layers is particularly valuable since its provision coincides with the grain filling period and has a high conversion efficiency into grain yield (Kirkegaard et al., 2007). Most of this increase in seed yield from water accessed late in the season from deeper soil layer waters is due to increases in the HI of the crop (Passioura and Angus, 2010), as also observed for the non-hybrid cultivar in the present study.

5. Conclusions

Biomass of a vigorous hybrid cultivar benefitted more from the 'CO$_2$ fertilisation effect' only with ample water, whereas under water limited conditions the non-hybrid cultivar showed greater e[CO_2]-stimulation of biomass and seed yield. Better balance between assimilate supply and sink capacity may enable a greater 'CO$_2$ fertilisation effect', but this balance additionally depends on water regime. With transpiring leaf area stimulated by e[CO_2] regardless of water treatments, smaller relative CO$_2$-effect on g_s under dry conditions lead to consistently greater water use under e[CO_2], whereas greater relative reduction of g_s under well-watered conditions offset greater transpiring leaf area to keep water use unchanged. Stimulation of root growth in deeper soil layers and the associated greater extraction of deeper soil water can support a greater 'CO$_2$ fertilisation effect'.

References

Ainsworth, E.A., Long, S.P., 2005. What have we learned from 15 years of free-air CO$_2$ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy properties and plant production to rising CO$_2$. New Phytol. 165, 351–371.

Allen, L.H., Kakani, V.G., Vu, J.C.V., et al., 2011. Elevated CO$_2$ increases water use efficiency by sustaining photosynthesis of water-limited maize and sorghum. J. Plant Physiol. 168, 1909–1918.

Aranjuelo, I., Sanz-Sáez, A., Jáuregui, I., et al., 2013. Harvest index, a parameter conditioning responsiveness of wheat plants to elevated CO_2. J. Exp. Bot. 64, 1879–1892.

Bahrami, H., Armstrong, R., Fitzgerald, G.J., et al., 2015. Can intra-specific differences in root traits of wheat increase nitrogen use efficiency (NUE) under elevated CO_2? In: Edwards, D., Oldroyd, G. (Eds.), Agriculture and Climate Change – Adapting Crops to Increased Uncertainty. Elsevier Science B.V., Amsterdam, pp. 109–110.

Benlloch-Gonzalez, M., Berger, J., Bramley, H., et al., 2014a. The plasticity of the growth and proliferation of wheat root system under elevated CO_2. Plant Soil 374, 963–976.

Benlloch-Gonzalez, M., Bochicchio, R., Berger, J., et al., 2014b. High temperature reduces the positive effect of elevated CO_2 on wheat root system growth. Field Crops Res. 165, 71–79.

Bishop, K.A., Betzelberger, A.M., Long, S.P., et al., 2015. Is there potential to adapt soybean (*Glycine max* Merr.) to future CO_2? An analysis of the yield response of 18 genotypes in free-air CO_2 enrichment. Plant Cell Environ. 38, 1765–1774.

Bourgault, M., James, A.T., Dreccer, M.F., 2016. Pot size matters revisited: does container size affect the response to elevated CO_2 and our ability to detect genotypic variability in this response in wheat? Funct. Plant Biol. 44, 52–61.

Bunce, J.A., 2016. Responses of soybeans and wheat to elevated CO_2 in free-air and open top chamber systems. Field Crops Res. 186, 78–85.

Burkart, S., Manderscheid, R., Weigel, H.J., 2004. Interactive effects of elevated atmospheric CO_2 concentrations and plant available soil water content on canopy evapotranspiration and conductance of spring wheat. Eur. J. Agron. 21, 401–417.

Burkart, S., Manderscheid, R., Wittich, K.P., et al., 2011. Elevated CO_2 effects on canopy and soil water flux parameters measured using a large chamber in crops grown with free-air CO_2 enrichment. Plant Biol. 13, 258–269.

Chaudhuri, U.N., Kirkham, M.B., Kanemasu, E.T., 1990. Root-growth of winter-wheat under elevated carbon-dioxide and drought. Crop Sci. 30, 853–857.

Christy, B., Tausz-Posch, S., Tausz, M., et al., 2018. Benefits of increasing transpiration efficiency in wheat under elevated CO_2 for rainfed regions. Glob. Change Biol. 24, 1965–1977.

FAOSTAT, 2018. Food and Agriculture Organization of the United Nations Statistics Division. Available at:. (Accessed 21 February 2018). http://www.fao.org/faostat/en/#home.

Faralli, M., Grove, I.G., Hare, M.C., et al., 2017. Rising CO_2 from historical concentrations enhances the physiological performance of *Brassica napus* seedlings under optimal water supply but not under reduced water availability. Plant Cell Environ. 40, 317–325.

Farooq, M., Gogoi, N., Barthakur, S., et al., 2017. Drought stress in grain legumes during reproduction and grain filling. J. Agron. Crop Sci. 203, 81–102.

Fischer, R.A., 2007. Understanding the physiological basis of yield potential in wheat. J. Agric. Sci. 145, 99–113.

Flexas, J., Medrano, H., 2002. Drought-inhibition of photosynthesis in C_3 plants: stomatal and non-stomatal limitations revisited. Ann. Bot. 89, 183–189.

Flexas, J., Bota, J., Galmés, J., et al., 2006. Keeping a positive carbon balance under adverse conditions: responses of photosynthesis and respiration to water stress. Physiol. Plant. 127, 343–352.

Franzaring, J., Högy, P., Fangmeier, A., 2008. Effects of free-air CO_2 enrichment on the growth of summer oilseed rape (*Brassica napus* cv. Campino). Agric. Ecosyst. Environ. 128, 127–134.

Franzaring, J., Weller, S., Schmid, I., et al., 2011. Growth, senescence and water use efficiency of spring oilseed rape (*Brassica napus* L. cv. Mozart) grown in a factorial combination of nitrogen supply and elevated CO_2. Environ. Exp. Bot. 72, 284–296.

Frenck, G., van der Linden, L., Mikkelsen, T.N., et al., 2011. Increased [CO_2] does not compensate for negative effects on yield caused by higher temperature and [O_3] in *Brassica napus* L. Eur. J. Agron. 35, 127–134.

Gallardo, M., Turner, N.C., Ludwig, C., 1994. Water relations, gas-exchange and abscisic-acid content of *Lupinus cosentinii* leaves in response to drying different proportions of the root-system. J. Exp. Bot. 45, 909–918.

Gray, S.B., Dermody, O., Klein, S.P., et al., 2016. Intensifying drought eliminates the expected benefits of elevated carbon dioxide for soybean. Nat. Plants 2, 16132.

Gregory, P.J., McGowan, M., Biscoe, P.V., 1978. Water relations of winter wheat: 2. Soil water relations. J. Agric. Sci. 91, 103–116.

Hess, L., Meir, P., Bingham, I.J., 2015. Comparative assessment of the sensitivity of oilseed rape and wheat to limited water supply. Ann. Appl. Biol. 167, 102–115.

Högy, P., Franzaring, J., Schwadorf, K., et al., 2010. Effects of free-air CO_2 enrichment on energy traits and seed quality of oilseed rape. Agric. Ecosyst. Environ. 139, 239–244.

Hunsaker, D.J., Kimball, B.A., Pinter, P.J., et al., 2000. CO_2 enrichment and soil nitrogen effects on wheat evapotranspiration and water use efficiency. Agric. For. Meteorol. 104, 85–105.

Hussain, M.Z., Vanloocke, A., Siebers, M.H., et al., 2013. Future carbon dioxide concentration decreases canopy evapotranspiration and soil water depletion by field-grown maize. Glob. Change Biol. 19, 1572–1584.

IPCC, 2013. Technical summary. In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Jin, Z.N., Ainsworth, E.A., Leakey, A.D.B., et al., 2018. Increasing drought and diminishing benefits of elevated carbon dioxide for soybean yields across the US Midwest. Glob. Change Biol. 24, E522–E533.

Johannessen, M.M., Mikkelsen, T.N., Jorgensen, R.B., 2002. CO_2 exploitation and genetic diversity in winter varieties of oilseed rape (*Brassica napus*); varieties of tomorrow. Euphytica 128, 75–86.

Kimball, B.A., 2016. Crop responses to elevated CO_2 and interactions with H_2O, N, and temperature. Curr. Opin. Plant Biol. 31, 36–43.

Kimball, B.A., LaMorte, R.L., Pinter, P.J., et al., 1999. Free-air CO_2 enrichment and soil

nitrogen effects on energy balance and evapotranspiration of wheat. Water Resour. Res. 35, 1179–1190.

Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO_2 enrichment. In: Donald, L.S. (Ed.), Advances in Agronomy. Academic Press, pp. 293–368.

Kirkegaard, J.A., Lilley, J.M., Howe, G.N., et al., 2007. Impact of subsoil water use on wheat yield. Aust. J. Agric. Res. 58, 303–315.

Laza, M.R., Peng, S., Arnel, S., et al., 2001. Higher leaf area growth rate contributes to greater vegetative growth of F1 rice hybrids in the tropics. Plant Prod. Sci. 4, 184–188.

Leakey, A.D.B., Ainsworth, E.A., Bernacchi, C.J., et al., 2009. Elevated CO_2 effects on plant carbon, nitrogen, and water relations: six important lessons from FACE. J. Exp. Bot. 60, 2859–2876.

Liao, M.T., Fillery, I.R.P., Palta, J.A., 2004. Early vigorous growth is a major factor influencing nitrogen uptake in wheat. Funct. Plant Biol. 31, 121–129.

Liu, H.J., Yang, L.X., Wang, Y.L., et al., 2008. Yield formation of CO_2 enriched hybrid rice cultivar Shanyou 63 under fully open-air field conditions. Field Crops Res. 108, 93–100.

Maaz, T., Wulfhorst, J.D., McCracken, V., et al., 2018. Economic, policy, and social trends and challenges of introducing oilseed and pulse crops into dryland wheat cropping systems. Agric. Ecosyst. Environ. 253, 177–194.

Madhu, M., Hatfield, J.L., 2013. Dynamics of plant root growth under increased atmospheric carbon dioxide. Agron. J. 105, 657–669.

Manderscheid, R., Weigel, H.-J., 2007. Drought stress effects on wheat are mitigated by atmospheric CO_2 enrichment. Agron. Sustain. Dev. 27, 79–87.

Manea, A., Leishman, M.R., 2014. Leaf area index drives soil water availability and extreme drought-related mortality under elevated CO_2 in a temperate grassland model system. PLoS One 9.

Manschadi, A.M., Christopher, J., Devoil, P., et al., 2006. The role of root architectural traits in adaptation of wheat to water-limited environments. Funct. Plant Biol. 33, 823–837.

Miralles, D.J., Slafer, G.A., 2007. Sink limitations to yield in wheat: how could it be reduced? J. Agric. Sci. 145, 139–149.

NOAA, 2018. National Oceanic and Atmospheric Administration. (Accessed 30 June 2018). https://www.esrl.noaa.gov/gmd/ccgg/trends/monthly.html.

Palta, J.A., Finery, I.R.P., Rebetzke, G.J., 2007. Restricted-tillering wheat does not lead to greater investment in roots and early nitrogen uptake. Field Crops Res. 104, 52–59.

Passioura, J.B., Angus, J.F., 2010. Improving productivity of crops in water-limited environments. In: In: Sparks, D.L. (Ed.), Advances in Agronomy, vol. 106. pp. 37–75.

Pinheiro, J., Bates, D., DebRoy, S., et al., 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-131.1. https://CRAN.R-project.org/package=nlme.

Pritchard, S.G., Prior, S.A., Rogers, H.H., et al., 2006. Effects of elevated atmospheric CO_2 on root dynamics and productivity of sorghum grown under conventional and conservation agricultural management practices. Agric. Ecosyst. Environ. 113, 175–183.

Qaderi, M.M., Reid, D.M., 2005. Growth and physiological responses of canola (*Brassica napus*) to UV-B and CO_2 under controlled environment conditions. Physiol. Plant. 125, 247–259.

Qaderi, M.M., Kurepin, L.V., Reid, D.M., 2006. Growth and physiological responses of canola (*Brassica napus*) to three components of global climate change: temperature, carbon dioxide and drought. Physiol. Plant. 128, 710–721.

R Core Team, 2017. R: A Language and Environment for Statistical Computing. Retrieved from. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ruhil, K., Sheeba, Ahmad, A., et al., 2015. Photosynthesis and growth responses of mustard (*Brassica juncea* L. cv Pusa Bold) plants to free air carbon dioxide enrichment (FACE). Protoplasma 252, 935–946.

Samarakoon, A.B., Muller, W.J., Gifford, R.M., 1995. Transpiration and leaf-area under elevated CO_2 effects of soil-water status and genotype in wheat. Aust. J. Plant Physiol. 22, 33–44.

Sinaki, J.M., Heravan, E.M., Rad, A.H.S., et al., 2007. The effects of water deficit during growth stages of canola (*Brassica napus* L.). Am. J. Agric. Environ. Sci. 2, 417–422.

Tausz, M., Tausz-Posch, S., Norton, R.M., et al., 2013. Understanding crop physiology to select breeding targets and improve crop management under increasing atmospheric CO_2 concentrations. Environ. Exp. Bot. 88, 71–80.

Tausz-Posch, S., Dempsey, R.W., Seneweera, S., et al., 2015. Does a freely tillering wheat cultivar benefit more from elevated CO_2 than a restricted tillering cultivar in a water-limited environment? Eur. J. Agron. 64, 21–28.

Uddin, S., Löw, M., Parvin, S., et al., 2018a. Water use and growth responses of dryland wheat grown under elevated [CO_2] are associated with root length in deeper, but not upper soil layer. Field Crops Res. 224, 170–181.

Uddin, S., Löw, M., Parvin, S., et al., 2018b. Elevated [CO_2] mitigates the effect of surface drought by stimulating root growth to access sub-soil water. PLoS One 13, e0198928.

van der Kooi, C.J., Reich, M., Löw, M., et al., 2016. Growth and yield stimulation under elevated CO_2 and drought: a meta-analysis on crops. Environ. Exp. Bot. 122, 150–157.

VanVuuren, M.M.I., Robinson, D., Fitter, A.H., et al., 1997. Effects of elevated atmospheric CO_2 and soil water availability on root biomass, root length, and N, P and K uptake by wheat. New Phytol. 135, 455–465.

Veihmeyer, F.J., et al., 1956. Soil moisture. In: Adriani, M.J., Aslyng, H.C., Burström, H. (Eds.), Pflanze und Wasser/Water Relations of Plants. Springer, Berlin, Heidelberg, pp. 64–123.

Wall, G.W., Brooks, T.J., Adam, R., et al., 2001. Elevated atmospheric CO_2 improved sorghum plant water status by ameliorating the adverse effects of drought. New Phytol. 152, 231–248.

Wall, G.W., Garcia, R.L., Wechsung, F., et al., 2011. Elevated atmospheric CO_2 and drought effects on leaf gas exchange properties of barley. Agric. Ecosyst. Environ.

144, 390–404.

Wasson, A.P., Richards, R.A., Chatrath, R., et al., 2012. Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J. Exp. Bot. 63, 3485–3498.

Watson, J., Zheng, B.Y., Chapman, S., et al., 2017. Projected impact of future climate on water-stress patterns across the Australian wheatbelt. J. Exp. Bot. 68, 5907–5921.

Wu, D.X., Wang, G.X., 2000. Interaction of CO_2 enrichment and drought on growth, water use, and yield of broad bean (*Vicia faba*). Environ. Exp. Bot. 43, 131–139.

Wu, D.X., Wang, G.X., Bai, Y.F., et al., 2004. Effects of elevated CO_2 concentration on growth, water use, yield and grain quality of wheat under two soil water levels. Agric. Ecosyst. Environ. 104, 493–507.

Wujeska-Klause, A., Bossinger, G., Tausz, M., 2015. Seedlings of two *Acacia* species from contrasting habitats show different photoprotective and antioxidative responses to drought and heatwaves. Ann. For. Sci. 72, 403–414.

Yang, L.X., Liu, H.J., Wang, Y.X., et al., 2009a. Impact of elevated CO_2 concentration on inter-subspecific hybrid rice cultivar Liangyoupeijiu under fully open-air field conditions. Field Crops Res. 112, 7–15.

Yang, L.X., Liu, H.J., Wang, Y.X., et al., 2009b. Yield formation of CO_2-enriched inter-subspecific hybrid rice cultivar Liangyoupeijiu under fully open-air field condition in a warm sub-tropical climate. Agric. Ecosyst. Environ. 129, 193–200.

Zhu, C., Xu, X., Wang, D., et al., 2015. An indica rice genotype showed a similar yield enhancement to that of hybrid rice under free air carbon dioxide enrichment. Sci. Rep. 5, 12719.